Drinking Water and Dietary Sources of Nitrate and Nitrite and Risk of Glioma

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Objective: *Dietary nitrite has been associated with increased glioma risk; however, drinking water nitrate has not been extensively evaluated.* ***Methods:*** *We conducted a population-based case–control study of adult glioma in Nebraska. Water utility nitrate measurements were linked to residential water source histories. We computed average nitrate exposure over a 20-year period. A food frequency questionnaire was used to assess dietary nitrate and nitrite.* ***Results:*** *Increasing quartiles of the average nitrate level in drinking water were not significantly associated with risk (adjusted odd ratios: 1.4, 1.2, 1.3). Risk was similar among those with both higher and lower intakes of vitamin C, an inhibitor of N-nitroso compound formation. Dietary nitrite intake was not associated with risk.* ***Conclusions:*** *Our study does not support a role for drinking water and dietary sources of nitrate and nitrite in risk of adult glioma.* (J Occup Environ Med. *2005;47:1260–1267*)

Nitrate is a precursor compound in the formation of N-nitroso compounds (NOC), many of which are potent animal carcinogens.[1] Of the two major types of NOC, nitrosamines and nitrosamides, the latter, and in particular the nitrosoureas, are strong nervous system carcinogens.[1,2]

NOC exposure can occur in certain work environments through use of tobacco products and some household products; however, the most widespread exposure to preformed NOC comes from dietary intakes of preserved meats.[3] Endogenous formation of NOC (nitrosation) from NOC precursors has been estimated to account for the 45–75% of exposure.[3] Using a test that measures nitrosation rates in humans,[4] two studies showed positive associations between drinking water nitrate ingestion and NOC excretion in human volunteers.[5,6] In another study, those with higher nitrate levels in their water supplies had increased blood levels of a marker of mutagenicity.[7] Drinking water can be the major source of nitrate exposure when levels approach the U.S. Maximum Contaminant Level (MCL) of 10 mg/L nitrate-nitrogen (nitrate-N).[8,9] Below the MCL, nitrate comes from dietary sources, primarily vegetables.

Exposure to nitrate through water consumption has not been systematically assessed in the U.S. population. However, monitoring data from agricultural states show increases in nitrate levels in ground water over the past 50 years caused mainly by agricultural activity.[10,11] Elevated nitrate in drinking water has been associated with an increased risk of brain cancer in an ecologic study[12]; however, another

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ecologic study[13] and a case–control study[14] found no association with higher nitrate levels in public water supplies. Most epidemiologic studies of drinking water nitrate and cancer have been ecological or have lacked historical information on nitrate exposure.[15] The formation of NOC is inhibited by the consumption of antioxidants found in fruits and vegetables,[16] indicating that dietary information should be considered in addition to the assessment of drinking water nitrate exposures.

We conducted a case–control study of adult glioma in eastern Nebraska, a state with elevated nitrate levels in ground water, to further evaluate the association of adult glioma with drinking water nitrate exposure. Dietary intakes of nitrate, nitrite, and nitrosation inhibitors also were assessed.

Materials and Methods

We conducted a population-based case–control study of adult glioma in the 66 counties of eastern Nebraska. The study population has been described previously.[17] Controls were selected randomly from controls in a previous population-based case–control study of hematopoietic cancers in eastern Nebraska[18] and were re-interviewed at the time of the case interviews. Simultaneously, we also enrolled a population-based series of adenocarcinomas of the stomach and esophagus.[19,20]

Cases were white men and women ages 21 or older who had been newly diagnosed with glioma (ICD-O codes 938-948) between July 1, 1988, and June 30, 1993. Cases were limited to white residents of eastern Nebraska during 1983–1986 because the control subjects were selected during that time period and other ethnic groups had been excluded because of expected small numbers. Cases from 1988 through 1990 were identified from the Nebraska Cancer Registry, whereas cases from 1991 through June 30, 1993, were identified by reviewing discharge diagnoses and pathology records at 11 hospitals in Omaha and Lincoln. From 1987–1988, 94% of the gliomas in the study area were treated in these hospitals. To insure that we captured all gliomas, we requested records for adult primary brain cancers (ICD-O Topology codes 191-191.9) from the Registry and participating hospitals. From the Registry, we also requested any records coded as gliomas (ICD-O Morphology codes 938-948.1), pineal tumors (codes 936), gangliogliomas (code 950.5), neurocytomas (950.6), and neuroblastomas from other and unspecified parts of the nervous system (ICD-O Morphology code 950.0 and Topology code 192).

Cases were reviewed by a neuropathologist (R.D.M.) to identify eligible cases (only gliomas) and to determine the histological subtype.[21] Of the eligible interviewed cases 87% were astrocytic (56% of all cases were glioblastoma multiforme), 8% were oligodendrogliomas, 2% ependymomas, 2% mixed gliomas, and 1% other unspecified gliomas. Of the 282 eligible cases, interviews were obtained for 251 (89%; 112 women and 139 men). Because of the severity of the disease, interviews were conducted with the next-of-kin or proxies for 76% of cases. The large majority of proxy respondents were either the spouse (62%) or other first-degree relatives (33%).

We selected a stratified random sample from the previous population-based control subjects as described previously.[17] We over-sampled living controls to provide more power for analyses by respondent type. For the youngest cases, we had insufficient numbers of control subjects; therefore, new controls born during the years 1967 through 1971 were identified from death certificates ($n = 20$, deaths in 1988–1993) and from random digit dialing ($n = 3$) by the same methods as in the earlier study.

Of the 606 eligible controls, 503 (83%) were successfully interviewed. The response rate in the original study was 87%, giving an overall control response rate of 75%. Five control subjects were excluded after the interview because of a reported previous diagnosis of a brain tumor, leaving 498 control subjects in the analysis. Among these, 60% of the interviews were conducted with proxies. Proxy respondents were primarily the spouse (45%) or other first-degree relatives (46%).

Telephone interviews were conducted during 1992 to 1994. Interviewers obtained information about agricultural exposures, occupational and residential histories, usual adult diet, tap water intake, and other factors. For each residence, the interviewer asked about the primary source of drinking water (public supply, private well, bottled water, other). Water source histories were collected from birth through the residence in 1985, the time period of the previous study.

We used a 48-item food-frequency questionnaire that was a modified version of the short Block Health Habits and History Questionnaire[22] with the addition of foods high in nitrate and nitrite. The nitrate and nitrite contents of the food items were determined from the literature.[23–27] Those who had missing or unknown intakes for >20% of the food items were excluded from the dietary analyses. We adjusted for energy intake by including total calories in the regression models as a continuous variable. Adjustment using the residual method[28] gave similar results. The results for dietary nitrate and nitrite intakes have been reported for the entire study population.[17] Here, we present results for the population in the drinking water analyses and we evaluate animal and plant sources of nitrite separately. We also estimated the contribution of drinking water and diet sources of nitrate to endogenous nitrite formation.

Nitrate levels in many Nebraska public water supplies (PWS) were measured in 1947 and periodically thereafter as previously described.[29] Nitrate levels were measured using the phenol disulfonic acid method[30] before the late 1970s and the cadmium reduction method[31] thereafter.

Nitrate levels in drinking water are expressed as mg/L nitrate-nitrogen (nitrate-N).

We had nitrate measurements for samples taken from the distribution system and at the groundwater wells. If available, we used the distribution measurements to calculate an annual mean nitrate level; otherwise, measurements from wells serving the utility in that year were averaged. For years with no nitrate measurements, we assigned an estimate based on the annual mean nitrate concentration for the PWS in neighboring years. We calculated a weighted average where the weights were 1.0, 0.75, 0.5, and 0.25 depending on whether the annual mean was 1 to 2, 3 to 4, 5 to 6, or 7 to 9 years, respectively from the year missing measurement data. No cases reported using bottled water as their primary drinking water source and use was very limited among controls (mean years ± SD, 0.14 ± 1.63). Nitrate levels were assumed to be zero in bottled water and years of bottled water use were included in the calculation of average PWS nitrate levels. We assigned an unknown nitrate level for communities outside of Nebraska, and for years in which the respondent reported using a private well.

Of the 236 Nebraska PWS included in these analyses, 73 had one or more nitrate measurement in the 1940s, 29 had data in the 1950s, 121 in the 1960s, and 122 in the 1970s. All but three PWS had monitoring data in the 1980s. Among cases and control subjects in the nitrate analyses, the total number of years with nitrate data and the number of measurements per decade (1940s to 1980s) was similar (data not shown).

Study participants who had used a private well in 1985 (63 cases, 72 controls) were asked to supply a tap water sample from that well. Those who agreed were sent a sampling kit and instructions. A total of 47 cases (75%) and 43 controls (60%) had samples analyzed. The nitrate analysis was done by the Nebraska Department of Health Laboratories using the cadmium reduction method.[31]

We estimated the odds ratio (OR) using logistic regression analysis. Ninety-five percent confidence intervals (CI) were computed using Wald intervals.[32] In all of the analyses of drinking water nitrate levels, the widths of the confidence intervals given are likely to be narrower than they would have been had more elaborate variance adjustment procedures been used.

Because nitrate monitoring data were limited before the mid-1960s, we restricted most analyses to individuals who used Nebraska PWS after 1964. To reduce misclassification by unknown nitrate levels, mainly because of the use of private wells, we restricted the analyses to those with nitrate information (actual or imputed data) for 70% or more of their person-years after 1964 (130 cases, 319 controls). Our principal measure of nitrate exposure was an individual's average nitrate exposure after 1964. We also evaluated the number of years from 1947 onward, that a PWS had nitrate levels ≥5 mg/L and 10 mg/L, the regulatory limit.

We calculated PWS nitrate exposure from 1965 through 1985, the time frame of the previous study resulting in a lag of 3 to 8 years between the exposure period and case diagnosis. To ensure a comparable exposure lag period for cases and controls in the PWS analyses, we excluded 100 (31%) controls who died before the case ascertainment period. We found no difference in the results; therefore, we present our findings with all 319 controls.

We computed water nitrate intake by multiplying the usual tap water intake (including coffee, tea, and other beverages made with tap water) by the average PWS nitrate level for those with known intakes of tap water and coffee (the major contributors to tap water intake). An estimated 5% of nitrate intake is endogenously converted to nitrite.[3] We estimated endogenous nitrite from water nitrate intake only because the antioxidants present in diet nitrate sources inhibit the formation of NOC. We summed preformed diet nitrite and endogenous nitrite from drinking water to give total nitrite intake.

All exposure estimates were adjusted for year of birth in four categories (<1915; 1915 to 1924, 1925 to 1939, 1940+), education (less than high school, high school, some college or vocational school, college graduate/postgraduate), and farming (ever live or work on a farm). The dietary analyses also were adjusted for a familial history of brain cancer, calories, carotenoids, and fiber. Dietary intake of vitamin C and smoking were evaluated for effect modification of the water nitrate association. We did the major analyses separately for self- and proxy-respondents and found no meaningful differences by respondent type; therefore, we present results adjusted for respondent type. We analyzed the largest histological subgroups of gliomas, glioblastoma multiforme, and other astrocytomas separately. The numbers of other histological subgroups were too few to evaluate separately. The results for glioblastoma multiforme and astrocytomas were very similar to those for all gliomas so only the results for all gliomas are presented.

Results

Characteristics of Persons Included in the Drinking Water Nitrate Analyses

A greater proportion of cases in the PWS analyses were women compared with the overall study population (Table 1). As expected, those in the water analysis group had used Nebraska public water supplies for more years and private wells for fewer years than the overall study population. Those in the PWS analysis tended to have more years of formal education; fewer cases and control subjects had ever lived or worked on a farm. Among men included in the PWS analysis, fewer cases had lived or worked on a farm compared with controls, whereas, the opposite was true in the study population overall. The analysis group and

TABLE 1
Characteristics of the Study Population Included in the Public Water Supply Nitrate Analysis and the Total Study Population*

	Population in PWS Analyses†		Overall Study Population	
	Controls (n = 319)	Cases (n = 130)	Controls (n = 498)	Cases (n = 251)
Men	180 (56.4)	60 (46.2)	283 (56.8)	139 (55.4)
Women	139 (43.6)	70 (53.8)	215 (43.2)	112 (44.6)
Year of birth				
Before 1915	104 (32.6)	13 (10.0)	158 (31.7)	32 (12.7)
1915–1924	80 (25.1)	38 (29.2)	121 (24.3)	73 (29.1)
1925–1939	78 (24.4)	44 (33.8)	116 (23.3)	74 (29.5)
1940 or later	57 (17.9)	35 (27.0)	103 (20.7)	72 (28.7)
Nebraska public water				
Median yr (IQR)	41 (28–54)	42 (31–53)	30 (14–46)	28 (7–44)
Nebraska private wells				
Median yr (IQR)	0 (0–20)	0 (0–12)	8 (0–32)	0 (0–30)
Unknown source				
Median yr (IQR)	5 (0–7)	0 (0–4.5)	6 (1–7)	0 (0–5)
Education				
<12 yr	84 (26.4)	15 (11.5)	151 (30.3)	44 (17.5)
High school	101 (31.8)	46 (35.4)	140 (28.1)	84 (33.5)
Some college, vocational technical school	81 (25.5)	40 (30.8)	116 (23.3)	68 (27.1)
College 4 yr/Postgraduate	52 (16.3)	29 (22.3)	87 (17.5)	55 (21.9)
Ever live/work on farm?				
Men				
Yes	101 (56.1)	28 (46.7)	179 (63.2)	93 (67.4)
No	79 (43.9)	32 (53.3)	104 (36.8)	45 (32.6)
Women				
Yes	85 (61.2)	31 (44.3)	143 (66.5)	60 (53.6)
No	54 (38.8)	39 (55.7)	72 (33.5)	52 (46.4)
First-degree relative with cancer				
None	137 (42.9)	58 (44.6)	230 (46.2)	103 (41.0)
Central nervous system cancer	7 (2.2)	6 (4.6)	12 (2.4)	10 (4.0)
Other cancer	146 (45.8)	57 (43.9)	214 (43.0)	119 (47.4)
Don't know	29 (9.1)	9 (6.9)	42 (8.4)	19 (7.5)
Respondent				
Self	125 (39.2)	29 (22.3)	198 (39.8)	61 (24.3)
Spouse	89 (27.9)	62 (47.7)	135 (27.1)	118 (47.0)
Daughter/son	65 (20.4)	30 (23.1)	104 (20.9)	50 (19.9)
Parent/sibling	22 (6.9)	6 (4.6)	33 (6.6)	12 (4.8)
Other relative	15 (4.7)	1 (0.8)	22 (4.4)	7 (2.8)
Other	3 (0.9)	2 (1.5)	6 (1.2)	3 (1.2)

*Percentages may not total 100 as the result of missing information.
†Population with 70% of person-yr 1965 to 1984 with public supply nitrate levels.

study population overall were similar with respect to a family history of cancer, the type of respondent providing the interview, and dietary intakes of nitrate, nitrite, carotenes and fiber (not shown).

Public Water Supply Nitrate Analyses

Average nitrate levels from 1965 to 1984 ranged from 0 to 12 mg/L. Among control subjects, the median was 2.58 and the interquartile range (IQR) was 2.38–4.32. Increasing quartiles of average nitrate were not associated with risk of glioma (Table 2). Results were similar for men and women (not shown). Water nitrate intake (mg nitrate-N/d) calculated by multiplying daily tap water intake by the average PWS nitrate level ranged from 0.2 to 39 mg/d (median 5.1; IQR 3.1–8.6) and was also not associated with risk (not shown).

A large number of participants were long-term residents of Omaha or Lincoln, the two largest cities. Long-term residents of Omaha were in the second quartile of average nitrate exposure, whereas long-term residents of Lincoln were in the fourth quartile. To determine whether residence in these cities might confound the nitrate association, we excluded long-term residents of these cities and re-evaluated the PWS nitrate association. The ORs were 1.0, 1.2, and 0.7 for increasing quartiles of the average nitrate level compared to the lowest quartile.

A total of 45 (35%) cases and 102 (32%) controls in the PWS nitrate

TABLE 2
Odds Ratios and 95% Confidence Intervals for Glioma, and the Average Nitrate Level in Nebraska Public Water Supplies, 1965 to 1984*

mg nitrate-N/L	Cases	Controls	OR† (95% CI)
<2.38	24	79	1.0
2.38–2.57	39	79	1.4 (0.7–2.7)
2.58–4.32	33	81	1.2 (0.6–2.3)
>4.32	34	80	1.3 (0.7–2.6)

*Includes those with 70% or more of their person-yr after 1964 with Nebraska public water supply nitrate estimates.
†ORs adjusted for age, gender, respondent type, education, and ever live/work on a farm.

analysis had 1 or more years of exposure to public supplies with a nitrate level ≥10 mg/L nitrate-N from 1947–1984 (Table 3). Compared with no exposure, there was no association between the years of exposure to drinking water nitrate at these levels and risk of glioma. Increasing years using a PWS with nitrate levels of ≥5 mg/L was associated with nonsignificant increasing risk up to 9 years, however there was no association among those exposed for 10 or more years.

We evaluated the risk of glioma with combined exposure to quartiles of the PWS average nitrate and usual vitamin C intake (≤median, >median; Table 4). There was no evidence of an interaction between drinking water nitrate and dietary vitamin C intake. ORs were nonsignificantly elevated in all categories of water nitrate and vitamin C intake compared to those with low water nitrate and high vitamin C intake. We obtained similar results when we evaluated years of exposure ≥5 mg/L nitrate-N. We also found no evidence of interaction by smoking status (current/past smokers and nonsmokers—not shown).

TABLE 3
Odds Ratios (OR) and 95% Confidence Intervals (CI) for Glioma and Year of Using a Nebraska Public Water Supply With ≥5 and ≥10 mg/L Nitrate, 1947 to 1984*

Years	Cases	Controls	OR† (95% CI)
≥10 mg/L			
0	85	217	1.0
1–8	25	54	1.1 (0.6–2.1)
9+	20	48	1.1 (0.6–2.0)
≥5 mg/L			
0	22	82	1.0
1–4	62	127	1.3 (0.7–2.5)
5–9	20	33	1.8 (0.8–4.1)
10+	26	77	1.1 (0.5–2.2)

*Includes those with 70% or more of their person-yr after 1964 with Nebraska public water supply nitrate estimates.
†Odds ratios adjusted for year of birth, gender, respondent type, education, and ever live/work on a farm.

Nitrite Intake From Diet and Drinking Water Sources

A total of 121 cases and 285 control subjects in the PWS nitrate analysis had adequate dietary information (<20% unknown food items). As was previously reported for the entire study population,[17] there was no association between dietary nitrate (not shown), dietary nitrite, and glioma risk among those in the PWS analysis (Table 5). Higher intake of plant nitrite was associated with a significantly increased risk; there was no association with animal sources. Total nitrite as the sum of dietary nitrite and endogenous nitrite from drinking water nitrate was not significantly associated with risk (Table 5). There was no evidence of an interaction between vitamin C intake and dietary nitrite or nitrite intake from diet and drinking water (not shown).

Private Well Nitrate Analyses

The nitrate levels measured in private wells ranged from below the detection limit (0.5 mg/L nitrate-N) to 67 mg/L nitrate-N (median among

TABLE 4
Odds Ratios (OR) and 95% Confidence Intervals (CI) for Glioma Associated With The Combined Average Nitrate Level In Nebraska Public Water Supplies (1965 to 1984) and Dietary Vitamin C Intake*

	Public Water Supply Nitrate Level (mg nitrate-N/L)			
Vitamin C mg/d	<2.36 Cases/Controls OR (CI)	2.38–2.57 Cases/Controls OR† (CI)	2.58–4.32 Cases/Controls OR (CI)	>4.32 Cases/Controls OR (CI)
113+	8/38	22/41	13/33	13/31
	1.0	2.4 (0.8–6.9)	2.0 (0.7–6.0)	2.1 (0.7–6.4)
<113	15/33	16/34	17/38	17/37
	2.3 (0.8–6.9)	1.6 (0.5–4.8)	1.7 (0.6–4.7)	2.0 (0.7–5.8)

*Includes those with 70% or more of their person-yr after 1964 with Nebraska public water supply nitrate estimates.
†ORs adjusted for year of birth, gender, respondent type, education, and ever live/work on a farm.

TABLE 5
Odds Ratio (OR) and 95% Confidence Intervals (CI) for Glioma and Intakes of Nitrite

mg/d	Cases/Controls	OR (CI)*
Preformed diet nitrite†		
<0.70	38/67	1.0
0.70 to <0.94	27/74	0.8 (0.4–1.7)
0.94–1.19	23/71	1.0 (0.4–2.3)
≥1.19	33/73	1.2 (0.5–3.2)
Diet nitrite from animal sources†		
<0.29	29/68	1.0
0.29 to <0.46	25/72	0.8 (0.4–1.6)
0.46–0.63	31/70	1.3 (0.6–2.7)
≥0.63	36/75	1.3 (0.6–2.9)
Diet nitrite from plant sources†		
<0.31	34/68	1.0
0.31 to <0.43	29/74	1.0 (0.5–2.1)
0.43–0.59	28/68	3.2 (1.2–8.3)
≥0.59	30/75	2.8 (1.0–8.2)
Preformed diet intake plus endogenous nitrite from water nitrate‡		
<1.4	21/70	1.0
1.4 to <2.1	43/71	1.8 (0.9–3.6)
2.1 to <3.3	28/71	1.1 (0.5–2.3)
≥3.3	28/71	1.1 (0.5–2.5)

*Odds ratios adjusted for year of birth, gender, respondent type, ever live/work on a farm, education, beta-carotene, fiber, calories.

†Includes those with 70% or more of their person-yr after 1964 with Nebraska public water supply nitrate estimates and adequate dietary information (excludes those with >20% unknown foods).

‡Includes those with 70% or more of their person-yr after 1964 with Nebraska public water supply nitrate estimates and adequate dietary and tap water information (excludes those with >20% unknown foods and unknown water/coffee intake).

controls 2.3; IQR 0.2–7.4; 90th percentile 16.6). There was no association between increasing tertiles of the nitrate level and glioma risk (not shown). We compared those with private well nitrate levels at or above 10 mg/L (16% of controls; 15% of cases) to those whose wells were lower but found no association with risk (OR = 1.2, 95% CI = 0.4–4.1). The small number of individuals prohibited the evaluation of effect modification.

We evaluated the duration of private well use in Nebraska and risk of glioma because private well users often have higher water nitrate exposure than those using public supplies. Results differed for men and women. Among men, there was a significantly 2.5-fold increase among those with 30 or more years using private wells for their drinking water (Table 6). Among women, duration of private well use was inversely associated with risk. We evaluated years of well use after 1964 (Table 6) because nitrate levels in many water supplies increased between the 1960s and 1980s.[29,33] Fifteen or more years of well use after 1964 was associated with a significant 3.1-fold increased risk among men but there was no association among women. Shallow wells (<100 feet) tend to have higher nitrate levels than deeper wells.[34] Only 17 (6.8%) cases and 98 (19.7%) control subjects had ever used a shallow well for their drinking water. We observed a pattern of risk similar to total years of well use; among men the OR for 30 or more years was elevated (OR = 1.7, 95% CI = 0.6–4.7) and among women the OR was inverse (OR = 0.4, 95% CI = 0.1–1.4).

TABLE 6
Odds Ratios (ORs) and 95% Confidence Interval (CI) for Glioma With Duration of Private Well Use in Nebraska

	All			Men			Women		
	Cases	Controls	OR (95% CI)*	Cases	Controls	OR (95% CI)†	Cases	Controls	OR (95% CI)†
Years of private well use in Nebraska‡									
0	105	178	1.0	48	110	1.0	57	68	1.0
1–9	12	26	0.6 (0.3–1.4)	8	16	1.1 (0.4–3.0)	4	10	0.2 (0.05–1.0)
10–19	27	52	0.8 (0.4–1.5)	10	24	0.9 (0.4–2.4)	17	28	0.5 (0.2–1.3)
20–29	18	56	0.6 (0.3–1.1)	13	30	1.0 (0.4–2.4)	5	26	0.2 (0.04–0.7)
30+	64	134	1.4 (0.8–2.5)	42	77	2.5 (1.2–5.4)	22	57	0.5 (0.2–1.3)
Years of private well use in Nebraska after 1964‡									
0	168	363	1.0	83	206	1.0	85	157	1.0
1–14	28	61	1.2 (0.7–2.0)	16	35	1.2 (0.6–2.6)	12	26	1.1 (0.4–2.5)
15+	55	74	2.3 (1.4–3.6)	40	42	3.1 (1.7–5.7)	15	32	1.3 (0.6–2.8)

*ORs adjusted for year of birth, gender, respondent type, ever live/work on a farm, and education.

†ORs adjusted for year of birth, respondent type, ever live/work on a farm, and education.

‡Excludes those with private well use outside of Nebraska.

Discussion

The average nitrate level in public drinking water supplies in Nebraska during a 20-year period was not associated with glioma risk. Likewise, years of exposure to nitrate at or above the MCL of 10 mg/L nitrate-N and half the MCL was not significantly associated with risk; the duration of exposure above the MCL generally was low.

Elevated nitrate in drinking water was associated with an increased risk of brain cancer in a small area ecologic study in Yorkshire, England[12]; however, another ecologic study in Canada[13] found no association. The highest average exposure quartile in the Yorkshire study (5.3 mg/L nitrate-N) was slightly higher than our highest quartile level. A case-control study in Germany[14] found no association between glioma risk and average nitrate levels in PWS that were similar to average levels in our study.

In our study, the nitrate level in private wells at the time of the interview was not associated with risk of glioma; however, a current well measurement may not reflect long-term exposure. Longer duration of use of Nebraska private wells, particularly after 1964, was associated with an elevated risk of glioma among men but not women. In our study population, the duration of private well use was strongly correlated with the duration of farming and it was not possible to adjust for farming duration. Occupational surveys indicate that farmers have a higher risk of brain cancer compared with the general population.[35] Residual confounding by factors associated with long-term farming may explain the increased risk we observed.

Our study does not support dietary nitrite intake as a risk factor for adult glioma risk. Plant sources of nitrite, which consist mainly of baked goods and cereals, would not be expected to preferentially result in increased NOC formation compared with animal sources. Therefore, the significant association for plant nitrite was unexpected. Further, nitrite intake which incorporated the contribution of drinking water nitrate to endogenous nitrite formation was also not associated with risk. We did not observe any evidence of an interaction between nitrite intake and vitamin C.

Other case–control studies that estimated dietary nitrite have found mixed results.[36–41] A study by Lee et al[40] found a significantly elevated risk among men with high nitrite intake and low vitamin C intake. A recent meta-analysis of nine studies evaluating cured meat found a pooled RR of 1.48 (95% CI = 1.20–1.83) for glioma among adults ingesting high levels of cured meat.[42] However, the authors concluded that the failure of most studies to adjust for total energy intake might have led to spurious positive associations in some studies.

Strengths of our analysis include the high response rates among cases and controls, a lifetime water source history, a historical nitrate database for public water supplies, our evaluation of effect modification by vitamin C intake and smoking, and our ability to estimate nitrite intake from diet and drinking water. Our analysis of community supply nitrate levels was limited to considering exposures that occurred over the course of approximately 20 years due to infrequent measurements before 1965. Our study had little power to evaluate risk at nitrate levels above the MCL because of the few study participants who were using a private well at the time of the interview and because of the infrequent exposure above this level among public water supply users.

Because of the high mortality rate among glioma cases, we conducted the majority of interviews with proxies, most of whom were spouses or other first-degree relatives. We found no substantial differences in our results by respondent type. We had both living and deceased control subjects in our study. Controls chosen from mortality files have been shown to have a poorer diet and a higher proportion of heavy drinkers and smokers compared to living persons of the same age[43] which would result in underestimation of the risk for these factors. However, these differences between living and deceased controls would be unlikely to affect our drinking water nitrate analyses. The dates-of-death of some deceased controls preceded the case ascertainment period by 3 to 5 years. Because of the longer time between the death date and interview date for some deceased controls compared with deceased cases, proxies for these controls may have had more difficulty in recalling information. However, excluding controls whose date-of-death preceded the case ascertainment period did not materially change our results.

Nitrosamines must be metabolized before they are biologically active, whereas nitrosamides are chemically unstable, acting at the site of formation.[44] Nitrosamides are particularly strong neurocarcinogens in animal studies when administered in utero.[2] However, the animal and epidemiologic evidence for the carcinogenicity of NOCs for adult glioma is less clear.

In summary, we found no association between drinking water nitrate and glioma risk. Our study had limited power to evaluate PWS nitrate levels greater than approximately 5 mg/L nitrate-N. Dietary nitrite intake was not associated with risk and incorporating the contribution of water and dietary nitrate to endogenous nitrite formation did not change our findings. Large studies in populations with higher exposures to drinking water nitrate and dietary nitrite are warranted to further evaluate the NOC hypothesis in relation to adult glioma.

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